

DATA EVALUATION RECORD
WHOLE SEDIMENT CHRONIC TOXICITY, MARINE INVERTEBRATES
NON-GUIDELINE

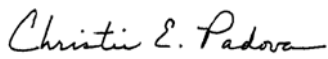
1. **CHEMICAL:** Permethrin PC code: 109701

2. **TEST MATERIAL:** Permethrin Purity: 97.7%

3. **CITATION:**

Authors: Picard, C.R.
Title: 28-Day Toxicity Test Exposing Estuarine Amphipods
(*Leptocheirus plumulosus*) to Permethrin Applied to
Sediment Following EPA Test Methods.
Study Completion Date: February 20, 2013
Laboratory: Smithers Viscient
790 Main Street
Wareham, MA 02571-1037
Sponsor: Consumer Specialty Products Association for the
Permethrin Data Group II Steering Committee/Joint Venture
1667 K Stree, NW, Suite 300
Washington, DC 20006
Laboratory Report ID: 13981.6106
MRID No.: 49063101
DP Barcode: 413721

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, CSS-Dynamac

Signature:  **Date:** 11/03/14

APPROVED BY: Teri S. Myers, Senior Scientist, CDM Smith.

Signature:  **Date:** 11/14/14

5. **APPROVED BY:**

Signature: **Date:**

6. **STUDY PARAMETERS:**

Age of Test Organism:	Neonates, >0.25 to <0.6 mm
Definitive Test Duration:	28 days
Study Method:	Static renewal
Type of Concentrations:	Mean-measured sediment (bulk and OC-normalized) and freely-dissolved pore

water

7. CONCLUSIONS:**Results Synopsis:****USING NEGATIVE CONTROL FOR COMPARISON**

Endpoint	Mean-measured Sediment, µg ai/kg	OC-normalized Sediment, µg ai/g OC	Freely-Dissolved Pore Water, ng ai/L
Survival	NOAEC: 280 LOAEC: 680 LC ₅₀ : 550 95% C.I.: 500 to 600	NOAEC: 13 LOAEC: 32 LC ₅₀ : 26 95% C.I.: 24 to 28	NOAEC: 47 LOAEC: 120 LC ₅₀ : 96 95% C.I.: 87 to 100
Growth	NOAEC: 97 LOAEC: 280 EC ₅₀ : 370 95% C.I.: 280 to 450	NOAEC: 4.6 LOAEC: 13 EC ₅₀ : 17 95% C.I.: 13 to 22	NOAEC: 17 LOAEC: 47 EC ₅₀ : 63 95% C.I.: 48 to 76
Reproduction	NOAEC: 38 LOAEC: 97 EC ₅₀ : 120 95% C.I.: 73 to 210	NOAEC: 1.8 LOAEC: 4.6 EC ₅₀ : 5.3 95% C.I.: 3.4 to 10	NOAEC: 6.5 LOAEC: 17 EC ₅₀ : 20 95% C.I.: 13 to 38

8. ADEQUACY OF THE STUDY:

A. Classification: This study [is/is not scientifically sound] and is classified as [acceptable/supplemental (quantitative)/supplemental (qualitative)/invalid].

B. Rationale: This study was conducted following guidance outlined in the “Methods for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-Associated Contaminants with the Amphipod *Leptocheirus plumulosus*.” (U.S. EPA, 2001, EPA/600/R-01/020), and does not fulfill any current U.S. EPA OCSPP data requirement.

C. Repairability: N/A

9. MAJOR GUIDELINE DEVIATIONS: None**10. SUBMISSION PURPOSE:** RR**11. MATERIALS AND METHODS:**

Stability of Compound Under Test Conditions: Sediment and pore water samples were analyzed for permethrin concentrations at 0, 14, and 28 Days (all levels). Concentrations remained predominantly associated with the sediment phase throughout the 28-day study, with mean-measured concentrations ranging from 75 to 89% of nominal levels. Freely-dissolved pore water concentrations were calculated by the study author and are reported in Table 1 of the DER.

Physicochemical properties of permethrin.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV adsorption	Not reported	
pKa	Not reported	
Kow	Not reported	

OECD requires water solubility, stability in water and light, pKa, P_{ow}, and vapor pressure of the test compound.

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
<u>Species</u> <i>Leptocheirus plumulosus</i>	Marine amphipod, <i>Leptocheirus plumulosus</i>
<u>Source</u> Laboratory cultures or commercial or government sources.	Laboratory cultures
<u>Acclimation Period/Culture Conditions</u> Periodic-renewal (two to three times weekly) culture system maintained at 20 to 25°C and under a 16-hour light/8-hour dark photoperiod. Amphipods are cultured in bins with a thin (<i>ca.</i> 1 to 1.5 cm) layer of clean, fine-grained sediment and at least 10 cm of clean, continuously-aerated dilution water (same as test water).	Amphipod cultures were maintained in plastic culture tubs with a layer of 0.25-mm sieved marine sediment and <i>ca.</i> 9 L of 20‰ seawater (same as used during testing). Measured water quality parameters 1 week prior to test initiation yielded a salinity of 20 to 22‰ and a temperature of 24°C.

Guideline Criteria	Reported Information
<p><u>Life Stage</u> Neonates: age-selected (<48 hours old) or size-selected (preferable): retained between 0.25-mm and 0.6-mm mesh screens.</p>	<p>Neonates: >0.25 to <0.6 mm</p> <p>The mean dry weight obtained from three, 20-neonate subsets (n=60 total) resulted in an initial dry weight of 0.039 mg dw per amphipod (used to determined growth rate at the end of the exposure).</p>
<p><u>Food</u> Finely-ground TetraMin® provided two or three times per week (in conjunction with media renewal). Other food sources (e.g., alga) are also acceptable.</p>	<p>Organisms were not fed during the acclimation period.</p>
<p><u>Pretest Mortality</u> Do not use if organisms appear unhealthy, discolored, or otherwise stressed (e.g., >20% mortality in 48-hr period preceding the test).</p>	<p>No mortality observed during the 48-hour period preceding the test.</p>

B. Test System

Guideline Criteria	Reported Information
<p><u>Type of Test System</u> Static-renewal</p> <p>Siphon off and replace 400 mL of overlying water three times per week (Monday, Wednesday, and Friday).</p>	<p>Static-renewal</p> <p>400 mL of overlying water was siphoned off and replaced three times per week. The replacement of overlying water was done so as not to disturb the sediment layer.</p>

Guideline Criteria	Reported Information
<p><u>Test Material(s)</u></p>	<p>Identity: permethrin IUPAC name: not reported CAS name: not reported CAS no.: 52645-53-1 Physical description: not reported Batch No.: PMPS000014 Purity: 97.7% (w:w) Storage: dark, room temperature</p>
<p><u>Stock Solutions</u></p>	<p>A 200 µg ai/mL (adjusted for purity) primary stock was prepared in acetone, and from this five individual dosing stock solutions were prepared at 4.10 to 160 µg ai/mL in acetone. All dosing stock solutions were clear and colorless with no visible undissolved test substance.</p>
<p><u>Source of dilution water (overlying water) and sediment</u> Clean seawater, natural or reconstituted water from the same source was used for culturing.</p> <p>Uncontaminated natural sediment, which has been press-sieved (0.25-mm). Sediments should be stored at 4°C in the dark and used within 2 to 8 weeks of collection.</p>	<p>Natural seawater (salinity 30 to 32‰ and pH 7.7 to 8.3) was pumped from the Cape Cod Canal, Bourne, MA (<i>ca.</i> 4 m offshore at a depth of approx. 0.5 m). The seawater was held in a re-circulating epoxy-coated concrete holding reservoir. Prior to use, the dilution water was adjusted with laboratory well water to a salinity of 19 to 21‰ and a pH of 7.0 to 8.1.</p> <p>Marine sediment (Batch No. 040412-M-3) was collected from Little Harbor Beach, Wareham, MA. The sediment was wet-pressed through a 0.25-mm sieve to remove large particles and amended with fine, industrial sand to obtain an organic carbon content within the appropriate range. Sediment was then flushed with dilution water for <i>ca.</i> 18 days.</p>

Guideline Criteria	Reported Information
<u>Does water support test animals without observable signs of stress?</u>	Yes
<u>Quality Of Water</u>	There were no apparent problems with water quality. The TOC of the overlying water was 1.9 and 1.0 mg/L for the months of August and September 2012, respectively.
<u>Water Temperature</u> Overall: 25(±2)°C Daily limits: 25(±3)°C	Overall range (daily meas.): 24 to 25°C Overall range (cont. meas.): 24 to 26°C
<u>pH</u> 7.0 to 9.0 pH units	Overall range: 7.2 to 8.1
<u>Dissolved Oxygen</u> Overall: ≥4.4 mg/L (60% saturation) Daily limits: ≥3.6 mg/L (50% saturation)	Overall range: 4.7 to 7.9 mg/L (>50% saturation)
<u>Salinity</u> Overall: 5(±2)‰ (if estuarine sediment; pore water 1 to 10‰) or 20(±2)‰ (if marine sediment; pore water >10 to 35‰) Daily limits: 5(±3)‰ or 20(±3)‰	Overall range: 20 to 21‰
<u>Ammonia</u>	Day 0: 4.1 to 4.4 mg/L as N Day 28: ≤0.13 mg/L as N
<u>Sediment Characterization</u> Grain size: >5% silt and clay to <85% clay Total organic carbon (TOC): 1.5 to 4%	Particle distribution – 85% sand, 10% silt, and 5% clay TOC – 2.1% Moisture content – 19.5% (at 1/3 bar) Percent solids – 57.16% pH – 6.7 Ammonia (pore water) – 44.0 mg/L (as nitrogen; bulk sediment prior to use)

Guideline Criteria	Reported Information
<u>Additional Sediment Analysis</u> Pore water ammonia should not exceed 60 mg/L at test initiation. Pore water salinity range of 1 to 35‰ during the study.	<u>Pore Water:</u> DOC: 24 to 54 mg/L TOC: 51 to 79 mg/L Salinity: 20 to 21‰ pH: 6.9 to 7.9 Temperature: 20 to 23°C Ammonia (as N): 30 to 38 mg/L on Day 0, 3.2 to 9.3 mg/L on Day 28
<u>Sediment Spiking</u>	<p>A jar-rolling technique was used to apply the test substance to the sediment. A 10-mL volume of the appropriate dosing stock solution (in acetone) was applied to 0.050 kg of fine silica sand in glass Petri dishes, and the solvent was allowed to completely evaporate (30 minutes).</p> <p>The dried treated sand was then added to 3.5 kg of wet sediment (2.0506 kg dw) in individual jars. Each jar was then rolled for 4 hours at room temperature at approx. 15 rpm. The jars were stored upright at 2 to 8°C during equilibration.</p>
<u>Sediment Conditioning</u>	<p>The treated sediments were allowed to equilibrate for a 2-week period in the refrigerator. Once weekly during the equilibration period and prior to addition to the exposure vessels, the jars were mixed on the rolling mill for an additional 2 hours at room temperature to ensure the sediment was homogeneous.</p>

Guideline Criteria	Reported Information
<p><u>Sediment and Overlying Water Into Test Chambers</u> 1-L glass chambers (10 cm id) containing 175 mL (<i>ca.</i> 2 cm) of sediment and <i>ca.</i> 725 mL of overlying water (total final volume of 900 mL).</p>	<p>Test systems were established on Day -1: 1000-mL glass vessels containing 175 mL (approx. 2-cm layer) of sediment and 725 mL of overlying water. The total overlying water plus sediment volume was maintained at <i>ca.</i> 900 mL.</p> <p>A turbulence reducer (modified plastic disk) was used to minimize the disruption of the sediment layer during the introduction of the overlying water.</p>
<p><u>Introduction of Test Organisms</u></p>	<p>Amphipods were impartially until each vessel contained 20 organisms. The test was initiated when each respective replicate contained 20 amphipods.</p>
<p><u>Aeration</u> Overlying sea water should be continuously aerated except when test organisms are being added. Aeration rate should be recorded daily in all chambers.</p>	<p>None reported</p>
<p><u>Photoperiod</u> 16-hour light/8-hour dark photoperiod using wide-spectrum fluorescent light with an intensity of 500 to 1000 lux.</p>	<p>16-hour light/8-hour dark photoperiod using fluorescent bulbs at an intensity range of 500 to 570 lux.</p>
<p><u>Solvents</u></p>	<p>Acetone, 10 mL per 2.0506 kg sediment (dw basis). The acetone was allowed to completely evaporate during the mixing procedure.</p> <p>Both solvent control and negative control groups were included in the study.</p>

C. Test Design

Guideline Criteria	Reported Information
<u>Duration</u> 28 days	28 days
<u>Nominal Sediment Concentrations</u> Control(s) and at least 5 test concentrations <u>Mean-Measured Sediment Concentrations</u>	0 (negative control), 0 (solvent control), 20, 50, 120, 310 and 780 µg ai/kg <2.1 (<LOQ, controls), 16, 38, 97, 280 and 680 µg ai/kg dw In addition, OC-normalized sediment concentrations and freely-dissolved pore water concentrations were calculated by the study author (see Table 1 and Reviewer's Comments).
<u>Range-Finding Test</u>	<u>Preliminary toxicity assessment</u> <ul style="list-style-type: none"> Two initial 28-day preliminary exposures were conducted but repeated due to poor control survival and variable data. Third preliminary study: 28-day static-renewal exposure (as described for definitive study) at nominal concentrations of 0 (negative and solvent controls), 0.50, 5.0, 50, 500 and 5000 µg ai/kg Five replicates per level, each containing 20 neonate amphipods <u>Survival</u> averaged 93 (negative control), 93 (solvent control), 91, 98, 91, 93 and 1%, respectively; statistically-significant difference at the 5000 µg/kg level (level excluded from further comparisons) <u>Growth rate</u> averaged 0.051 (negative control), 0.057 (solvent control), 0.048, 0.048, 0.052 and 0.029 mg per day, respectively; statistically-significant difference at the 500 µg/kg level

Guideline Criteria	Reported Information
	<p>compared to the control</p> <ul style="list-style-type: none"> • <u>Reproduction</u> averaged 10 (negative control), 8 (solvent control), 8, 8, 8 and 2 offspring per female, respectively; statistically-significant difference at the 500 µg/kg level compared to the control • Overall NOAEC of 50 µg ai/kg
<p><u>Number of Test Organisms</u> 20 organisms per test chamber are recommended. Five replicates per treatment should be used.</p>	<p>120 amphipods per level, with 20 amphipods per replicate vessel and six biological replicates per level</p> <p>An additional five replicates (with amphipods) were maintained for chemical analysis and pore water quality measurements.</p>
<p><u>Feeding</u> Three times per week after water renewal.</p> <p><u>Recommended rates</u> Days 0 to 13: 20 mg TetraMin® per chamber Days 14 to 28: 40 mg TetraMin® per chamber</p>	<p>The amphipods were fed a flaked fish food suspension (10 mg/mL) three times per week following renewal of the overlying water.</p> <p>Days 0 to 13: 2.0 mL suspension per vessel Days 14 to 27: 4.0 mL suspension per vessel</p>
<p><u>Monitoring the test</u> Condition and activity of amphipods should be observed at each water renewal interval (i.e., three times per week).</p>	<p>Test vessels were observed daily for mortality and abnormal behavior.</p>

Guideline Criteria	Reported Information
<p><u>Overlying Water Parameter Measurements</u></p> <p>Daily temperature in water bath or test/dummy chamber – daily min/max is recommended. Temperature should also be measured in at least one replicate per treatment at study initiation, termination, and preceding water renewal intervals (three times weekly during the study).</p> <p>Salinity, DO, and pH should be measured in at least one replicate per treatment at study initiation, termination, and preceding water renewal intervals (three times weekly during the study).</p> <p>Total ammonia should be measured on Days 0 and 28 in one replicate per treatment.</p>	<p>Temperature was recorded in each replicate vessel on Days 0 and 28, and in one alternating replicate from each level on Days 1 to 27. Temperature was also continuously monitored in an auxiliary vessel in the water bath.</p> <p>Salinity, DO, and pH were measured in each replicate vessel on Days 0 and 28, and in one alternating replicate from each level on Days 1 to 27.</p> <p>Ammonia (as nitrogen) was recorded on Days 0 and 28 from a composite sample collected for each level.</p>
<p><u>Pore Water Parameter Measurements</u></p> <p>Total ammonia, salinity, temperature, and pH of pore water from surrogate containers on Days 0 and 28.</p>	<p>Dissolved organic carbon (DOC), total organic carbon (TOC), ammonia (as nitrogen), salinity, temperature, and pH of pore water were determined for each level from archived pore water samples on Day 1 (24-hr storage) and from surrogate vessels on Day 28 (see Reviewer's Comments).</p>

Guideline Criteria	Reported Information
Chemical Analysis	<p>Concentrations of permethrin were determined in sediment and pore water on Days 0, 14 and 28. Sediment and pore water were isolated using centrifugation (30 minutes at <i>ca.</i> 10,000 <i>g</i>). Overlying water was not analyzed (see Reviewer's Comments).</p> <p>All aqueous and sediment samples were analyzed for permethrin concentration using gas chromatography with mass selective detection with negative chemical ionization (GC/MS/NCI).</p>

12. REPORTED RESULTS:**A. General Results**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	<p>Yes. This study was conducted in compliance with the GLP standards of the U.S. EPA (40 CFR, Part 160) with the following exceptions: routine food, water and sediment screening analyses. However, analyses were conducted following standard validated methods.</p>

Guideline Criteria	Reported Information
<u>Control Criteria</u> Minimum mean control survival of 80%	Negative control – 73% (see Reviewer's Comments) Solvent control – 86%
Were growth and reproduction measurable in all control replicates?	Yes
<u>Percent Recovery of Chemical</u>	Sediment: 79.0 to 118% (n=9) Aqueous: 98.3 to 119% (n=8; excludes one outlier of 857% at the 0.0300 µg ai/kg level on Day 14) Based upon QC samples fortified and analyzed with set of samples.
<u>Data Endpoints</u> - Survival - Reproduction - Growth rate	- Survival - Reproduction - Growth rate
Raw data included?	Yes, sufficient

Effects DataTable 1. Summary of permethrin effects on *Leptocheirus plumulosus*.

Toxicant Concentration				Mean Survival (% \pm SD)	Mean Growth Rate per Amphipod per Day (mg \pm SD)	Mean Offspring per Surviving Amphipod \pm (No. \pm SD)
Nominal ($\mu\text{g ai/kg}$)	Mean- Measured Sediment ($\mu\text{g ai/kg}$)	OC- Normalized Sediment ($\mu\text{g ai/g OC}$) ^(a)	Freely- Dissolved Pore Water (ng ai/L) ^(a)			
Neg control	<LOQ ^(a)	---	---	73 \pm 11	0.046 \pm 0.0078	12.4 \pm 2.30
Sol control	<LOQ	---	---	86 \pm 15	0.051 \pm 0.0055	10.7 \pm 4.17
20	16	0.76	2.7	83 \pm 11	0.050 \pm 0.012	12.8 \pm 6.01
50	38	1.8	6.5	82 \pm 10	0.052 \pm 0.0064	14.3 \pm 3.30
120	97	4.6	17	93 \pm 10	0.048 \pm 0.0053	7.45 \pm 3.27**
310	280	13	47	86 \pm 15	0.031 \pm 0.0054*	3.90 \pm 1.49*
780	680	32	120	28 \pm 12*	0.011 \pm 0.0057 ^(c)	0 \pm 0 ^(c)

^(a) See Reviewer's Comments for calculation methods.

^(b) The LOQ was 2.0 to 2.1 $\mu\text{g ai/kg}$ for sediment samples.

^(c) Excluded from statistical analysis due to the effect on survival.

* Significantly-significant difference ($p < 0.05$) compared to the negative control and solvent control (Dunnett's Multiple Comparison Test).

** Significantly-significant difference ($p < 0.05$) compared to the negative control (Dunnett's Multiple Comparison Test).

Toxicity Observations: Following 28 days of exposure, survival averaged 73 and 86% in the negative and solvent control levels, respectively, compared to 83, 82, 93, 86 and 28% in the mean-measured 16, 38, 97, 280 and 680 $\mu\text{g ai/kg}$ treatment levels, respectively. The difference was statistically-significant compared to both the negative and solvent controls at the 680 $\mu\text{g ai/kg}$ level (Dunnett's Multiple Comparison Test, $p < 0.05$), and this level was excluded from further statistical analysis. Using mean-measured sediment concentrations and comparison to the negative control data, the 28-day LC_{50} (with 95% C.I.) was 550 (500 to 600) $\mu\text{g ai/kg}$, and the NOAEC and LOAEC were 280 and 680 $\mu\text{g ai/kg}$, respectively. Adjusted for the organic carbon (OC) content of the sediment (i.e., 2.1%), the NOAEC and LOAEC for 28-day survival were 13 and 32 $\mu\text{g ai/g OC}$, respectively, and the LC_{50} (with 95% C.I.) was 26 (24 to 28) $\mu\text{g ai/g OC}$. In terms of freely-dissolved pore water concentrations, the NOAEC, LOAEC and LC_{50} (with 95% C.I.) values were 47, 120 and 96 (87 to 100) ng ai/L, respectively.

The mean growth rate was 0.046 and 0.051 mg/amphipod/day for the negative and solvent control groups, respectively, compared to 0.050, 0.052, 0.048, 0.031 and 0.011

mg/amphipod/day for the mean-measured 16, 38, 97, 280 and 680 $\mu\text{g ai/kg}$ treatment levels, respectively. The difference was statistically-significant compared to both the negative and solvent controls at the 280 $\mu\text{g ai/kg}$ level (Dunnett's Multiple Comparison Test, $p < 0.05$); the 680 $\mu\text{g ai/kg}$ level was excluded from statistical analysis. Using mean-measured sediment concentrations and comparison to the negative control data, the EC_{50} (with 95% C.I.) was 370 (280 to 450) $\mu\text{g ai/kg}$, and the NOAEC and LOAEC were 97 and 280 $\mu\text{g ai/kg}$, respectively. Adjusted for the organic carbon (OC) content of the sediment (i.e., 2.1%), the NOAEC and LOAEC for 28-day growth were 4.6 and 13 $\mu\text{g ai/g OC}$, respectively, and the EC_{50} (with 95% C.I.) was 17 (13 to 22) $\mu\text{g ai/g OC}$. In terms of freely-dissolved pore water concentrations, the NOAEC, LOAEC and EC_{50} (with 95% C.I.) values were 17, 47 and 63 (48 to 76) ng ai/L , respectively.

The average number of offspring per surviving female was 12.4, 10.7, 12.8, 14.3, 7.45, 3.90 and 0 for the negative control, solvent control, and mean-measured 16, 38, 97, 280 and 680 $\mu\text{g ai/kg}$ treatment levels, respectively. Differences were statistically-significant compared to the negative control at the 97 and 280 $\mu\text{g ai/kg}$ levels (Dunnett's Multiple Comparison Test, $p < 0.05$); the 680 $\mu\text{g ai/kg}$ level was excluded from statistical analysis due to a significant effect on survival. Compared to the solvent control, the difference was only significant at the 280 $\mu\text{g ai/kg}$ level. Using mean-measured sediment concentrations and comparison to the negative control data, the EC_{50} (with 95% C.I.) was 120 (73 to 210) $\mu\text{g ai/kg}$, and the NOAEC and LOAEC were 38 and 97 $\mu\text{g ai/kg}$, respectively. Adjusted for the organic carbon (OC) content of the sediment (i.e., 2.1%), the NOAEC and LOAEC for 28-day reproduction were 1.8 and 4.6 $\mu\text{g ai/g OC}$, respectively, and the EC_{50} (with 95% C.I.) was 5.3 (3.4 to 10) $\mu\text{g ai/g OC}$. In terms of freely-dissolved pore water concentrations, the NOAEC, LOAEC and EC_{50} (with 95% C.I.) values were 6.5, 17 and 20 (13 to 38) ng ai/L , respectively.

No sub-lethal or behavioral effects were noted among the amphipods in any of the treatment replicates.

B. Statistical Results

At study termination (Day 28), data obtained on amphipod survival, growth, and reproduction were statistically analyzed. Analyses were performed with CETIS™ Version 1.8.4.20 (2011) statistical software. Percent survival data were transformed (e.g., arcsine square-root percentage) prior to analysis. Results were provided in terms of mean-measured sediment concentrations (bulk and OC-normalized) and calculated freely-dissolved pore water concentrations (see Reviewer's Comments for calculation methods).

An Equal Variance Two-Sample t-Test was used to compare the performance of the negative control and solvent control groups. Regardless of the results, treatment-level data were compared to both control groups.

For all endpoints, the data were tested for normality using the Shapiro-Wilks' Test, and for homogeneity of variance using Bartlett's Test. All endpoints met both assumptions and treatment-level data were compared to both the negative and solvent control groups using Dunnett's Multiple Comparison Test. NOAEC and LOAEC values were assigned based on significance. All statistical conclusions were made at the 95% level of certainty except in the basic assumption tests (i.e., Shapiro-Wilks' Test and Bartlett's Test), in which the 99% level of certainty was applied.

The LC₅₀ with associated 95% confidence intervals (C.I.) was determined using the Trimmed Spearman-Kärber method within CETIS™. The EC₅₀ for reproduction and growth (with associated 95% C.I.) were calculated using the linear interpolation method within CETIS™.

Most sensitive endpoint(s): reproduction

USING NEGATIVE CONTROL FOR COMPARISON

Endpoint	Methods	Mean-measured Sediment, µg ai/kg	OC-normalized Sediment, µg ai/g OC	Freely-Dissolved Pore Water, ng ai/L
Survival	Dunnett's Multiple Comparison Test	NOAEC: 280 LOAEC: 680 LC ₅₀ : 550 95% C.I.: 500 to 600	NOAEC: 13 LOAEC: 32 LC ₅₀ : 26 95% C.I.: 24 to 28	NOAEC: 47 LOAEC: 120 LC ₅₀ : 96 95% C.I.: 87 to 100
Growth		NOAEC: 97 LOAEC: 280 EC ₅₀ : 370 95% C.I.: 280 to 450	NOAEC: 4.6 LOAEC: 13 EC ₅₀ : 17 95% C.I.: 13 to 22	NOAEC: 17 LOAEC: 47 EC ₅₀ : 63 95% C.I.: 48 to 76
Reproduction		NOAEC: 38 LOAEC: 97 EC ₅₀ : 120 95% C.I.: 73 to 210	NOAEC: 1.8 LOAEC: 4.6 EC ₅₀ : 5.3 95% C.I.: 3.4 to 10	NOAEC: 6.5 LOAEC: 17 EC ₅₀ : 20 95% C.I.: 13 to 38

USING SOLVENT CONTROL FOR COMPARISON

Endpoint	Methods	Mean-measured Sediment, µg ai/kg	OC-normalized Sediment, µg ai/g OC	Freely-Dissolved Pore Water, ng ai/L
Survival	Dunnett's Multiple Comparison Test	NOAEC: 280 LOAEC: 680 LC ₅₀ : 540 95% C.I.: 500 to 590	NOAEC: 13 LOAEC: 32 LC ₅₀ : 25 95% C.I.: 23 to 28	NOAEC: 47 LOAEC: 120 LC ₅₀ : 95 95% C.I.: 87 to 100
Growth		NOAEC: 97 LOAEC: 280 EC ₅₀ : 360 95% C.I.: 260 to 430	NOAEC: 4.6 LOAEC: 13 EC ₅₀ : 17 95% C.I.: 12 to 20	NOAEC: 17 LOAEC: 47 EC ₅₀ : 60 95% C.I.: 43 to 72
Reproduction		NOAEC: 97 LOAEC: 280 EC ₅₀ : 130 95% C.I.: 66 to 230	NOAEC: 4.6 LOAEC: 13 EC ₅₀ : 5.8 95% C.I.: 3.3 to 11	NOAEC: 17 LOAEC: 47 EC ₅₀ : 22 95% C.I.: 12 to 37

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer verified the results for survival, dry weight growth rate, and reproduction using CETIS 1.8.7.12 with backend settings implemented by EFED on 03/25/14. The negative and solvent control groups were compared using a two-tailed, equal variance t-test; no significant differences were detected and subsequent comparisons to the treated groups were made using only the negative control. The data were tested for normality and homogeneity of variances using Shapiro Wilks and Bartlett's/Levene's tests, respectively. The NOAEC and LOAEC values were determined using Dunnett's test.

Endpoint	Methods	NOAEC (µg ai/kg)	LOAEC (µg ai/kg)
Survival	Dunnett's test	280	680
Growth	Dunnett's test	97	280
Reproduction	Dunnett's test	38	97

Most sensitive endpoint: Reproduction

14. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study author's when using the negative

control for comparison; reproduction was the most sensitive endpoint.

This study followed guidance outlined in the “Methods for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-Associated Contaminants with the Amphipod *Leptocheirus plumulosus*” (U.S. EPA, 2001, EPA/600/R-01/020), and does not fulfill any current U.S. EPA OPPTS data requirement. Minimum control requirements established by this method include $\geq 60\%$ survival in each replicate, $\geq 80\%$ mean survival, and measurable growth and reproduction in all replicates. In this study, overall survival in the negative control group was 73%, with individual replicate survival rates of 85, 75, 65, 55, 80 and 80% which does not fulfill the minimum control survival validity requirement. The study author reported that due to the difficult nature of the test method and the clear response in the treatment groups, the study was considered to be scientifically acceptable. It was further noted that statistical analyses were compared to both the negative control and solvent control in order to provide the most conservative estimate of effect levels for the various endpoints.

In addition to mean-measured sediment concentrations, results were reported (by the study author) in terms of OC-normalized sediment and freely-dissolved pore water concentrations (Table 1). The organic carbon content of the natural sediment was 2.1%. Mean-measured sediment concentrations were normalized using the following equation:

$$C_{\text{sed OC}} = (C_{\text{sed}}/F_{\text{OC}}) \times 0.001$$

where:

C_{sed} = concentration in bulk sediment ($\mu\text{g ai/kg dw sediment}$)

$C_{\text{sed OC}}$ = OC-normalized concentration in sediment ($\mu\text{g ai/g OC}$)

f_{OC} = sediment OC content (fraction)

Pore water concentrations were measured at 0, 14 and 28 Days by chemical analysis following liquid-liquid extraction and results were provided in Table 4 of the study report. As the results represented total test substance, i.e., included both the freely-dissolved fraction and the fraction associated with the dissolved organic matter in the pore water, the freely-dissolved fraction was also estimated by the study author using the following equation:

$$C_{\text{free}} = (1000 \times C_{\text{sed OC}}/K_{\text{OC}})$$

where:

$C_{\text{sed OC}}$ = OC-normalized concentration in sediment ($\mu\text{g ai/g OC}$)

C_{free} = freely dissolved pore water concentration (ng ai/L)

K_{OC} = organic carbon partitioning coefficient

The sediment and aqueous method validations were conducted prior to initiation of the definitive test and established average recoveries of $103 \pm 5.73\%$ from artificial sediment and $84.9 \pm 12.3\%$ from freshwater. Conditions and procedures used throughout the analysis of the exposure solutions and QC samples during this study were similar to those used in the method validation studies.

Overlying water was not analyzed during this study due to the pyrethroids' strong affinity to sediment (i.e., high K_{OC} values) and regular renewal of the overlying water.

Pore water quality determinations were measured on Day 1 in archived pore water samples that were generated at test initiation. It was reported that since results were within typical ranges and pore water quality is not expected to change significantly over a 24-hour storage time period, this deviation did not have a negative impact on the results of the study.

Definitive test dates were August 2 to September 5, 2012.

15. REFERENCES:

- ASTM. 2002. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.
- Bousfield, E.L. 1973. Shallow Water Gammaridean Amphipoda of New England. Ithaca, NY: Cornell University Press. Pp. 312.
- Ditsworth, G.R., D.W. Schults, and J.K.P. Jones. 1990. Preparation of Benthic Substrates for Sediment Toxicity Testing. *Environmental Toxicology and Chemistry*. Vol. 9, pp. 1523-1529.
- European Commission. 2000. Residues: guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414. working document. Directorate General Health and Consumer Protection. SANCO/3029/99 rev. 4, 11/07/00.
- Ives, M. 2011. CETIS, Comprehensive Environmental Toxicity Information System™, User's Guide. Tidepool Scientific Software, McKinleyville, CA.
- Laskowski, D.A. 2002. Physical and chemical properties of pyrethroids. In: *Reviews of environmental contamination and toxicology*. Pub. Springer, Heidelberg, Germany. Vol. 174, pp. 49-170.
- U.S. EPA. 40 CFR, Part 160. Federal Insecticide, Fungicide, and Rodenticide Act. Good Laboratory Practices Standards; Final Rule. Office of the Federal Register, National Archives and Records Administration. U.S. Government Printing Office, Washington, DC.
- U.S. EPA. 2001. Methods for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-Associated Contaminants with the Amphipod *Leptocheirus plumulosus*. Office of Research and Development. Washington, DC. EPA/600/R-01/020.
- U.S. EPA. 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Forth Edition. EPA-821-R-02-013. Office of Water (4303T), U.S. Environmental Protection Agency, Washington, DC.